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Focus on Molecules: Rod photoreceptor cGMP-gated cation channel

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1. Structure

The rod photoreceptor cGMP-gated cation (rod CNG) channel is an essential component of phototransduction and also may play a critical role in maintenance of photoreceptor outer segment structural integrity. It is a non-selective cation channel that belongs to the superfamily of voltage-gated channels, and the subfamily of cyclic nucleotide gated channels (Kaupp and Seifert, 2002). This channel is composed of a heterotetramer consisting of three α -subunits (encoded by the CNGA1 locus; also called A subunit) and one β -subunit (encoded by the CNGB1 locus; also called the B subunit; Genbank IDs for Murine and Human mRNA: α -subunit 34328042, 71143140; β -subunit 28386104, 4502918; uniprot IDs for Murine and Human proteins are: α -subunit P29974, P29973; β -subunit Q9UMG2, Q80XL8) arranged around a central pore. The α - and β -subunits are the products of separate genes whose loci are designated CNGA1 and CNGB1. The CNGB1 locus is complex encoding multiple alternatively spliced transcripts that are expressed in several diverse tissues (Ardell et al., 2000). For example, an olfactory tissue specific transcript encodes a β -subunit variant that is required for normal channel function of the olfactory cyclic nucleotide gated channel.

As shown in Fig. 1, the core structure of the α - and β -subunits consists of six transmembrane domains, a pore loop region between domains 5 and 6, and a cGMP binding domain (CNBD). Additional, unique features of the β -subunit are a glutamic acid and proline-rich extension at the N-terminus (GARP region) and two modulatory Ca^{2+} /CaM binding domains (CaM-1 and CaM-2). The GARP region is also expressed in the form of two shorter soluble proteins called GARP-1 and GARP-2, both the products of alternative splicing (Ardell et al., 2000). GARP-2 is more abundant than the β -subunit, and both GARP proteins are exclusively localized to the rod outer segment. The extremely low abundance of GARP-1 has discouraged analysis of its possible function(s). Both GARP-2 and the GARP region of the β -subunit interact with the disk membrane protein peripherin/*rds* (Poetsch et al., 2001), and GARP-2 binds tightly and stoichiometrically to rod photoreceptor cGMP phosphodiesterase (PDE6; EC 3.1.4.35) in the absence of light (see below). Recent knockout studies of the CNGB1 locus indicate that the interaction between peripherin/*rds* (Uniprot ID P23942) and the β -subunit establishes a physical link between the disk and plasma membranes that may be critical to maintenance of the structural integrity of the rod outer

segment. The α -subunit binds tightly to the $\text{Na}^+/\text{Ca}^{2+}/\text{K}^+$ exchanger (NCKX1; Uniprot ID O60721), however, the significance of this interaction is currently unknown.

2. Function

The primary function of the rod channel is to regulate Na^+ and Ca^{2+} ion flow into the rod outer segment in a light dependent manner (Kaupp and Seifert, 2002; Matulef and Zagotta, 2003). The binding of Ca^{2+} in the channel pore is critical for gating and ion selectivity. Ca^{2+} binds in a voltage dependent manner to a highly conserved specific glutamic acid residue in the pore of all CNG channel α -subunits (corresponds to ³⁶⁵E in human rod CNG channel α -subunit), facilitating a small single channel conductance and an improved signal-to-noise ratio. Although the α - and β -subunits have the same basic structure, there are important functional differences. The α -subunit can form functional homomeric channels upon heterologous expression whereas the β -subunit cannot. In heterologous expression systems the β -subunit confers physiological properties on the channel complex that are more like the native rod channel such as flickery single channel gating, greater sensitivity to block by *l*-*cis*-diltiazem, higher affinity to cAMP and modulation by Ca^{2+} /calmodulin binding. Rod CNG channel is non-selectively permeable to most cations; however, under physiological conditions about 90% of the current is carried by Na^+ and 10% by Ca^{2+} . It can be opened cooperatively by cAMP or cGMP but shows very high affinity for cGMP and only a fraction of the current is carried by cAMP.

The cascade of events triggering channel closure initiates with light activation of the receptor, rhodopsin which leads to a rapid reduction of cytoplasmic cGMP mediated by rod PDE6 hydrolysis. The reduced cGMP levels lead to release of cGMP from rod CNG channel, subsequent channel closure, resulting in a transient hyperpolarization of the membrane potential. This signal is relayed to the bipolar cells through a reduction of glutamate release at the photoreceptor synapse. Intracellular Ca^{2+} levels fall about 10-fold as the NCKX1 exchanger continues to extrude Ca^{2+} . Lowered Ca^{2+} stimulates cGMP synthesis by guanylate cyclase (GC1; EC 4.6.1.2), which in turn opens the channel, thus restoring the dark current. Unlike the olfactory channel, rod CNG channel does not desensitize in continuous presence of the ligand but is modulated by several mechanisms. During high intracellular Ca^{2+} levels, Ca^{2+} /CaM binds to the CaM-1 binding domain of the β -subunit disrupting its interaction with the C-terminus of the α -subunit. Ca^{2+} /CaM modulation of rod CNG channel may be important in light adaptation and as a feedback mechanism to lower the affinity of the channel to cGMP in the dark. This process may be mediated by growth factors such as the retinal paracrine factor, IGF-1 by triggering a signalling cascade that lead to dephosphorylation of specific tyrosine residues (corresponds to ⁵⁰⁰Y and ⁹⁷⁷Y in the human α - and β -subunits, respectively).

A possible functional role for GARP-2 has recently been elucidated. The protein binds to PDE6 in the dark stabilizing the binding of the enzyme's inhibitory γ -subunits. This could serve to reduce system noise in the absence of light facilitating the increased sensitivity needed to allow single photon detection, a unique property of the rod photoreceptor cell.

3. Disease involvement

Mutations in the CNGA1 and CNGB1 loci encoding the α - and β -subunits cause autosomal recessive retinitis pigmentosa (arRP). Several CNGA1 mutations have been identified that generate null alleles, or produce truncated non-functional proteins or proteins that are improperly targeted to the plasma membrane (OMIM 123825). Two mutations in CNGB1 were shown to co-segregate with arRP (OMIM 600724). These are a frameshift mutation that truncates the last 28 amino acids and a substitution mutation of a conserved glycine in the CNBD that may interfere with cGMP binding to the β -subunit. *Cngb1* knockout mouse

models indicate that the β -subunit is required for proper expression and/or targeting of rod CNG channel to the outer segment. In these KO mice α -subunit expression is severely reduced and the photoresponse is absent or greatly attenuated. The mice exhibit a progressive retinal degeneration with similarities to human arRP. One KO that is a null for β -subunit expression in all tissues may affect olfactory function, an effect that is not observed in a rod photoreceptor specific *Cngb1* null mouse.

4. Future studies

Since the discovery of a cyclic nucleotide gated channel in the rod outer segments in 1985, tremendous progress has been made in understanding the function of the channel in phototransduction. There are still several aspects of the tuning of channel activity that remain to be determined. Structure/function relationships cannot be fully determined until a high resolution three dimensional structure can be elucidated. Until recently, studies on the rod CNG channel have focused on the ion channel function of the molecule which is commonly expressed in alternatively spliced isoforms in both photoreceptors and olfactory sensory neurons. While both the olfactory and rod CNG channel require a β -subunit subunit, only the rod channel has the unique N-terminal GARP region extension. The importance of this region in the β -subunit and the related soluble GARP proteins in channel function and outer segment structural integrity is an important area of recent focus. Determining the functional significance of the interaction between the channel and the NCKX1 exchanger, the two molecules that regulate Ca^{2+} flow into and out of the outer segment is also a critical area of investigation.

Acknowledgments

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References

- Ardell MD, Bedsole DL, Schoborg RV, Pittler SJ. Genomic organization of the human rod photoreceptor cGMP-gated cation channel beta-subunit gene. *Gene*. 2000; 245:311–318. [PubMed: 10717482]
- Kaupp UB, Seifert R. Cyclic nucleotide-gated ion channels. *Physiol. Rev.* 2002; 82:769–824. [PubMed: 12087135]
- Matulef K, Zagotta WN. Cyclic nucleotide-gated ion channels. *Annu. Rev. Cell. Dev. Biol.* 2003; 19:23–44. [PubMed: 14570562]
- Poetsch A, Molday LL, Molday RS. The cGMP-gated channel and related glutamic acid-rich proteins interact with peripherin-2 at the rim region of rod photoreceptor disc membranes. *J. Biol. Chem.* 2001; 276:48009–48016. [PubMed: 11641407]

Fig. 1.

Secondary structure of rod CNG channel α - and β -subunits. Both the α - and β -subunits have a common core structure that consists of six transmembrane domains, a pore region between the fifth and sixth domain and a cGMP binding domain (CNBD) in the carboxy-terminus. The α -subunit is post-translationally modified by cleavage of 94 amino acids from its amino-terminus (N-terminal cleavage) and undergoes N-linked glycosylation between transmembrane segments 5 and 6. The β -subunit has a glutamic acid and proline-rich segment (GARP Region) at the amino-terminus and two calmodulin binding domains (CaM-1 and CaM-2).